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Analytical Methods

In-house validation of a method for determination of silver nanoparticles in chicken meat based on asymmetric flow field-flow fractionation and inductively coupled plasma mass spectrometric detection

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ABSTRACT

Nanomaterials are increasingly used in food production and packaging, and validated methods for detection of nanoparticles (NPs) in foodstuffs need to be developed both for regulatory purposes and product development. Asymmetric flow field-flow fractionation with inductively coupled plasma mass spectrometric detection (AF^4-ICP-MS) was applied for quantitative analysis of silver nanoparticles (AgNPs) in a chicken meat matrix following enzymatic sample preparation. For the first time an analytical validation of nanoparticle detection in a food matrix by AF^4-ICP-MS has been carried out and the results showed repeatable and intermediately reproducible determination of AgNP mass fraction and size. The findings demonstrated the potential of AF^4-ICP-MS for quantitative analysis of NPs in complex food matrices for use in food monitoring and control. The accurate determination of AgNP size distribution remained challenging due to the lack of certified size standards.

1. Introduction

With the increasing use of nanotechnology in food and consumer products, there is a need for accurate and precise detection and characterization methods for nanoparticles (NPs) in complex matrices. Asymmetric flow field-flow fractionation (AF^4) coupled to inductively coupled plasma mass spectrometry (ICP-MS) is a highly promising method for this purpose (Dubascoux et al., 2010; von der Kammer, Legros, Larsen, Loeschner, & Hofmann, 2011). In (Linsinger et al., 2013) a generic approach for the validation of methods for detection and quantification of nanoparticles in food samples was described. It was concluded that validation of methods for detecting and quantifying NPs in food must answer three questions: (1) Are there *nano*particles in the sample (size identity), (2) if yes, what kind of particles (chemical identity) and (3) how much nanomaterial is in the sample (mass or number fraction). The use of spiked samples for validation studies was recommended and a number of rules were derived, including the use of non-agglomerated particles with known properties (i.e. particle size distribution and concentration) for spiking and the analysis of the spiked samples as quickly as possible to avid changes of the particles (Linsinger et al., 2013).

In a previous report, silver nanoparticle (AgNPs) spiked chicken meat was studied as a relevant model system to investigate the potential of AF^4-ICP-MS for detection and characterization of inorganic NPs in a complex food matrix (Loeschner et al., 2013a).

AgNPs were selected as an example of inorganic NPs because they are presently one of the most frequently used nanomaterials in products related to food, such as food storage containers and dietary supplements (nanotechproject.org, 2011). Chicken meat was chosen as an example of a complex food matrix, which illustrated a scenario where AgNPs may migrate from a bacteriostatic food contact material into meat. Recent studies showed that a fraction of Ag was released from food storage containers in the form of AgNPs (Echegoyen & Nerín, 2013; Goetz et al., 2013).

Before analyzing AgNPs in chicken meat, an AF^4 method was developed and optimized for the aqueous suspension of the AgNPs (Loeschner et al., 2013b). Later the same type of AgNPs was incorporated into chicken meat and a sample preparation method based on enzymatic digestion was developed (Loeschner et al., 2013a).

Here we present the results of an in-house validation study which evaluates the performance of the AF^4-ICP-MS method for AgNPs in chicken meat. The method parameters selectivity,

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linearity/working range, trueness/recovery, precision and limit of detection/limit of quantification were assessed following as close as possible the recommendations given in (Linsinger et al., 2013). In contrast to usual analytical methods, not only the presence and amount of a substance needed to be determined, but also the size of the particles to determine whether they are nanoparticles or not.

2. Experimental

2.1. Standards and reagents

Ultrapure water (18.2 MΩ/cm), which was obtained from a Millipore Element apparatus (Millipore, Milford, MA, USA), was used throughout the work. Polyvinylpyrrolidone (PVP)-stabilized AgNPs in aqueous suspension (NGAP NP Ag-2103) were purchased from Nanogap (Milladoiro, Spain) and characterized by the Institute for Reference Materials and Measurements (IRMM) as detailed elsewhere (Loeschner et al., 2013b). Briefly, the nominal size stated by the supplier was 42 ± 10 nm. The mass fraction of Ag determined by inductively coupled plasma optical emission spectrometry (ICP-OES) was 197.4 ± 0.6 μg/g (Loeschner et al., 2013a). The Ag mass fraction in filtrates obtained by ultrafiltration (cut-off 5 kDa) was 0.38 ± 0.04 μg/g. PVP K10 was used as steric stabilizer at a concentration of 3 μg/g. The zeta potential of the AgNPs was −37.9 ± 1.0 mV (pH 7). Lean chicken meat paste was produced by the Institute for Reference Materials and Measurements (IRMM) on the basis of 6 kg of fresh chicken breast filet obtained from a local butcher (Geel, Belgium). After cutting, freezing with liquid nitrogen and cryo-milling the material was allowed to thaw. The resulting product appeared as a homogenous meat paste. The meat paste was mixed with deionized water in a ratio 2:1 (m/m).

Additionally, a potential reference material of AgNP containing meat paste (referred to as "NanoLyse13") was produced by mixing meat paste with diluted AgNP suspension (NGAP NP Ag-2103) in a ratio 2:1 (m/m) to achieve a final nominal Ag mass concentration of 0.1 mg/g. The Ag mass fraction in NanoLyse13 samples was 105 ± 4 μg/g as determined by k0-neutron activation analysis. All samples were filled into 2 mL Nunc™ plastic cryo-vials and shock-frozen above liquid nitrogen. Dispatch was done on dry ice and the samples were stored at −80 °C: NanoLyse13 samples were stored for 10 months before analysis.

For the enzymatic digestion the commercial protease Proteinase K from *Engyodontium album* was used (Sigma–Aldrich St. Louis, MO, USA). ReagentPlus sodium dodecyl sulfate (SDS) with ⩾98.5% purity and sodium azide (NaN_3) with ⩾98% purity were purchased from Sigma–Aldrich (St. Louis, MO, USA). Nitric acid (67–69%) of PlasmaPURE quality and single element PlasmaCAL standards of Ag and rhodium (Rh, used as internal standard) at 1 mg/mL were obtained from SCP Science (Quebec, Canada). AF^4 carrier liquid was produced by dissolving ammonium bicarbonate NH_4HCO_3 (ReagentPlus, Sigma–Aldrich, St. Louis, MO, USA) in ultrapure water to a final concentration of 0.5 mM and adjusting the pH to 7.4 by adding nitric acid. As accumulation wall in the AF^4 channel polyether sulfone (PES) membranes (Nadir®, Lot no. 213150) with a molecular weight cut-off of 10 kDa were used and purchased from Wyatt Technology (Dernbach, Germany). For size calibration of the AF^4 channel 40 nm Nanosphere™ polystyrene nanoparticles (PSNPs) with an average hydrodynamic particle diameter of d_h = 41 ± 1.8 nm (NIST™ traceable size standard) from Thermo Fischer Scientific (Fremont, CA, USA) were used.

2.2. Sample preparation procedure

The detailed sample preparation procedure has been described earlier (Loeschner et al., 2013a). Briefly, a portion of 0.25 g thawed blank chicken meat paste was spiked with a volume of 125 μL (low level), 250 μL (medium level) or 375 μL (high level) of the AgNP suspension to achieve AgNP mass fractions in the samples of 65.8, 98.7, and 118.4 μg/g. An Eppendorf Multipette® Xstream with a 500 μL combitip was used for spiking (accuracy 0.483% and precision 0.468% for pipetting volume of 100 μL). The mixture was vortexed for 1 min at 2500 rpm and 5 mL of the Proteinase K solution (3 mg/mL Proteinase K in 50 mM NH_4HCO_3 buffer at pH 7.4 containing 5 mg/mL SDS and 0.2 mg/mL NaN_3) were added. The mixture was incubated at 37 °C in a water bath using continuous stirring for 40 min. A volume of 10 μL was injected into the AF^4 channel. Blank meat without AgNPs was processed using the same procedure. Instead of AgNPs an equal volume of ultrapure water was added. NanoLyse13 samples were thawed and carefully agitated with a disposable polypropylene spatula before addition of the Proteinase K solution. A probe sonicator (Microson XL 2000, QSonica, LLC) operating at 20 kHz, and equipped with a P1-probe of 3.2 mm diameter, 127 mm length and a maximum amplitude of 180 μm was applied for probe sonication of the NanoLyse13 samples after enzymatic digestion. The electrical input power was set to 5 W (the point shortly before foaming of the sample occurred). The volume of the sample was 2.5 mL.

2.3. Sample analysis

The instrumentation as well as the separation and detection methods are described in more detail elsewhere (Loeschner et al., 2013a, 2013b). The AF^4 system used in this study consisted of an Agilent 1200 series autosampler (G1329A), a high performance liquid chromatography pump (G1311A) (Agilent Technologies, Santa Clara, CA, USA), an Eclipse™ 3 AF^4 flow control module, and a short channel-type AF^4 separation channel (Wyatt Technology Europe GmbH, Dernbach, Germany) with a 350 μm spacer. The AF^4 separation program used a detector flow rate of 1.0 mL/min and a constant cross flow rate of 1.0 mL/min (40 min elution with cross flow after sample injection and focusing). Retention times t_r were converted to d_h by calibration using PSNPs (for details see (Loeschner et al., 2013b)). For each analytical sequence, two PSNP samples were analyzed.

Following separation by AF^4, a series 1200 diode array detector (Agilent G1315A, DAD) was used to record absorption spectra in the wavelength range of 191–949 nm (steps of 10 nm) every two seconds. As the final detector in the hyphenated system an ICP-MS instrument (ICP-MS 7500ce, Agilent Technologies, Japan) was used. External calibration with internal standardization (10 ng/mL Rh added to carrier liquid and standards) was applied to quantify the Ag mass concentration in the eluate following AF^4 separation (for details see (Loeschner et al., 2013a)). The ^{107}Ag and ^{103}Rh signal intensities were recorded. An external calibration curve was established based on analysis of six concentration levels of certified silver standard in 2% v/v HNO_3, which were introduced off-line using a peristaltic pump. The external mass concentration standard curve was established in the beginning and in the end of the each sequence. Integration of selected peaks or of the whole fractogram resulted in the Ag mass per peak or total recovered Ag mass, respectively (ng). Finally, the Ag mass fraction in the chicken meat samples was calculated taking the injection volume, the dilution factor and the density of the analyzed sample (=1 g/cm^3) into account.

2.4. Transmission electron microscopy (TEM)

The pristine AgNP suspension as well as the enzymatically digested meat sample containing AgNPs were diluted twice with ultrapure water. A volume of 10 μL of the diluted suspension was applied to a 200 mesh Formvar/carbon-coated copper grid. The